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Hyaline cartilage cells outperform mandibular condylar cartilage cells in a TMJ fibrocartilage tissue engineering application

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Summary

Objective: To compare temporomandibular joint (TMJ) condylar cartilage cells *in vitro* to hyaline cartilage cells cultured in a three-dimensional (3D) environment for tissue engineering of mandibular condylar cartilage.

Design: Mandibular condylar cartilage and hyaline cartilage cells were harvested from pigs and cultured for 6 weeks in polyglycolic acid (PGA) scaffolds. Both types of cells were treated with glucosamine sulfate (0.4 mM), insulin-like growth factor-I (IGF-I) (100 ng/ml) and their combination. At weeks 0 and 6, cell number, glycosaminoglycan (GAG) and collagen content were determined, types I and II collagen were visualized by immunohistochemistry and GAGs were visualized by histology.

Results: Hyaline cartilage cells produced from half an order to a full order of magnitude more GAGs and collagen than mandibular condylar cartilage cells in 3D culture. IGF-I was a highly effective signal for biosynthesis with hyaline cartilage cells, while glucosamine sulfate decreased cell proliferation and biosynthesis with both types of cells. *In vitro* culture of TMJ condylar cartilage cells produced a fibrous tissue with predominantly type I collagen, while hyaline cartilage cells formed a fibrocartilage-like tissue with types I and II collagen. The combination of IGF and glucosamine had a synergistic effect on maintaining the phenotype of TMJ condylar cells to generate both types I and II collagen.

Conclusion: Given the superior biosynthetic activity by hyaline cartilage cells and the practical surgical limitations of harvesting cells from the TMJ of a patient requiring TMJ reconstruction, cartilage cells from elsewhere in the body may be a potentially better alternative to cells harvested from the TMJ for TMJ tissue engineering. This finding may also apply to other fibrocartilages such as the intervertebral disc and knee meniscus in applications where a mature cartilage cell source is desired.

Key words: Glucosamine sulfate, Fibrocartilage tissue engineering, Hyaline cartilage, Mandibular condylar cartilage, TMJ.

Introduction

Temporomandibular joint (TMJ) disorders resulting from arthritis, ankylosis, traumas, internal derangement and/or other afflictions can ravage TMJ structures that have limited capacity for regeneration. Tissue engineering may provide an ideal solution, especially when severe degeneration occurs[1,2]. Although our long-term goal is to regenerate the whole mandibular condyle of the TMJ, the specific focus of the current study was the condylar cartilage, where cell sources and signals were investigated. The purpose of this study was to compare the behavior of mandibular condylar cartilage cells and ankle (hyaline) cartilage cells under the regulation of insulin-like growth factor-I (IGF-I) and glucosamine sulfate.

The composition and structure of mandibular condylar cartilage (henceforth referred to as TMJ condylar cartilage) differ from hyaline cartilage. Hyaline cartilage cells are chondrocytes, and hyaline cartilage can be divided into four zones: superficial, middle, deep and calcified. In contrast, TMJ condylar cartilage has both fibrocartilaginous and hyaline-like character, with a thin proliferative zone that separates the fibrocartilaginous fibrous zone at the surface from the hyaline-like mature and hypertrophic zones below. Unlike hyaline cartilage, TMJ condylar cartilage contains predominantly fibroblasts and collagen I in the superficial fibrous zone[3–5]. Undifferentiated mesenchymal cells are distributed in the proliferative zone, serving as a cell reservoir to provide cells for the fibrous zone and underlying zones[6–10]. The underlying mature and hypertrophic zones include differentiated chondrocytes and the collagen is both types I and II[3–5,11]. From the standpoint of embryonic origin, TMJ condylar cartilage is categorized as a secondary cartilage formed by periosteum or endosteum, while hyaline cartilage is a primary cartilage, which precedes bone formation. TMJ condylar cartilage falls under the classification of fibrocartilage, with a strong presence of both collagen types I and II[5,11]. However, hyaline cartilage contains predominately collagen type II in all zones[12]. These contrasting structures make it imperative to investigate and compare the responses of TMJ condylar cartilage cells and hyaline cartilage cells to biological signals and biodegradable scaffolds.

Previous tissue engineering studies of TMJ condylar cartilages utilized cells from the condyle itself[13], hyaline cartilage cells[14], fibroblasts[15,16] and stem cells[13,17–24] as cell sources. An early attempt conducted by Weng *et al.*[14] seeded bovine hyaline chondrocytes from the forelimbs onto the surface of a polyglycolic acid/polylactic acid (PGA/PLA) scaffold as a cartilage layer. Hollister and colleagues[15,16] also seeded bone morphogenetic protein (BMP)-transduced fibroblasts into scaffolds. In both studies, cartilage formation was

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observed after implantation by Safranin-O staining. Furthermore, bone marrow and umbilical cord mesenchymal stromal cells[13,17–24] were introduced to TMJ tissue engineering, demonstrating the feasibility of creating a tissue-engineered mandibular condyle in the form of two stratified layers. In these studies, the positive immunohistochemical (IHC) staining for types I and II revealed the existence of a fibrocartilage. Although most previous studies used stem cells for TMJ tissue engineering[13,17–24], an advantage that mature cells possess over various types of stem cells is the absence of the variable of differentiation and reduced possibility of metaplasia. This study thus aimed to compare TMJ condylar cartilage cells to hyaline cartilage cells for tissue engineering applications.

Although the cell behavior of TMJ condylar cartilage and hyaline cartilage has been extensively investigated, there are only three studies comparing the respective cell types, performed in newborn rat explant culture by a single group[5,25,26]. These studies revealed that basic fibroblast growth factor (bFGF), IGF-I and transforming growth factor beta (TGF-β) stimulated higher cell proliferation in the TMJ condylar explants than in the femoral head explants. IGF-I increased glycosaminoglycan (GAG) formation, while bFGF and TGF-β decreased GAG production in both types of cartilage. It must be noted that these studies used newborn rat condyles, which grew faster than mature condyles[27], whereas in the current study, cells were isolated from mature porcine mandibular condylar and ankle cartilage. In a two-dimensional (2D) environment using mature cells[28], IGF-I promoted cell proliferation and biosynthesis for both types of cells. Therefore, based on both explant and monolayer cell culture, IGF-I was chosen in our three-dimensional (3D) study to regulate cell growth and biosynthesis.

Glucosamine has been scarcely tested in tissue engineering applications, with a handful of recent studies. One recent investigation revealed that glucosamine hydrochloride up-regulated matrix production at concentrations between 0 and 2 mM[29]. Glucosamine sulfate has been shown *in vitro* to promote aggrecan production[30,31], and to inhibit matrix degrading protein production in chondrocytes[30]. In our 2D monolayer culture[28], glucosamine sulfate also demonstrated an ability to significantly promote GAG and collagen production and to outperform growth factors in certain instances (between 0 and 0.4 mM) for both TMJ condylar cartilage cells and ankle cartilage. Consequently, besides IGF-I at 100 ng/ml, glucosamine at 0.4 mM was adopted and the synergistic effects of IGF-I and glucosamine were also investigated in the current study.

Non-woven PGA scaffolds have been broadly exploited in both hyaline[32–34] and fibrocartilage cartilage[35–37] tissue engineering, due to its good biocompatibility and biodegradability. Our previous study[13] also demonstrated that non-woven PGA meshes did support proliferation and biosynthesis of TMJ condylar cartilage cells as evidenced by a significant increase in cell number and the presence of types I and II collagen and GAGs throughout the constructs. Therefore, in this study, non-woven PGA scaffolds were seeded with porcine cartilage cells from mature TMJ condylar cartilage and hyaline cartilage from ankles. Exogenous signals including glucosamine sulfate (0.4 mM) and IGF-I (100 ng/ml) and their combination were employed to regulate cell proliferation and extracellular matrix production. The comparison between these two types of cells provides critical steps in demonstrating that hyaline cartilage cells may be a promising mature cell source for TMJ condylar cartilage tissue engineering.

Materials and methods

CELL HARVESTING

Hog heads and ankles (Chester white breed, female, age 8 months) were acquired from Winchester Meat Processing (Winchester, KS, USA). The TMJ was first removed *en bloc* with capsule intact and placed into 100% ethanol for 20 min. The TMJ was scrubbed using a sterile iodine pad, then the joint capsule was broken and the disc was removed in a sterile manner with a scalpel in a tissue culture hood. TMJ condylar cartilage was removed by chopping cartilage from the surface of the TMJ condyle, washed with sterile phosphate buffered saline (PBS), minced, and digested for 24 h in 2 mg/ml type II collagenase (394 U/mg; Worthington Biochemical; Lakewood, NJ, USA). The cell solution was centrifuged and cells were resuspended in a cell culture medium, consisting of Dulbecco's Modified Eagle medium (Invitrogen; Carlsbad, CA, USA), 10% fetal bovine serum (FBS; Gemini; West Sacramento, CA, USA), 25 μg/ml ascorbic acid (Sigma; St. Louis, MO, USA), 1% non-essential amino acids (Invitrogen; Carlsbad, CA, USA) and 1% penicillin–streptomycin–fungizone (Invitrogen; Carlsbad, CA, USA). Cells were fed every other day until confluent. The confluent cell population was trypsinized and labeled as passage 1. The procedure to obtain cartilage cells from ankles was similar to harvesting TMJ condylar cartilage cells, although more straightforward. Briefly, skin was removed, the joint was sterilized using an iodine pad, the joint capsule was broken, and cartilage was obtained and minced. After digestion, the cells were resuspended, cultured in the flasks, and labeled as passage 1.

CELL SEEDING AND GROWTH FACTOR INCORPORATION

A non-woven PGA mesh (50 mg/cc; Synthecon; Houston, TX, USA) was punched into disc-shape scaffolds with a 5 mm diameter and 2 mm thickness, and then sterilized with ethylene oxide. After sterilization, the scaffolds were aired in sterilization pouches under a fume hood for 1 day, then wetted with sterile filtered ethanol for 5 min and two washes of sterile PBS. The scaffolds were then soaked in the cell culture medium for 1 day and then removed for cell seeding. P1 cells were seeded on the PGA scaffolds at 50 million cells/ml of scaffold. Highly concentrated cell solution (500 μl) was dispensed slowly onto scaffolds in 24-well plates, which were set on an orbital shaker at 150 rpm for 12 h. After 12 h, 500 μl of fresh medium was added. After 24 h, an additional 1 ml of medium was added and cells were allowed to attach statically in the medium for another day. After allowing 2 days for seeding and attachment (recorded as day 1), the medium was replaced by 2 ml of either fresh medium or the medium supplemented with either growth factor or glucosamine. There were a total of eight groups in this study, including the TMJ condylar and hyaline cartilage cells treated by IGF (100 ng/ml; Pepro Tech; Rocky Hill, NJ, USA), D-glucosamine 6-sulfate (0.4 mM, corresponding to 100 mg/ml; Sigma; St. Louis, MO, USA) and their combination (100 ng/ml IGF and 0.4 mM glucosamine) and their respective controls (cultured in fresh medium without IGF and glucosamine treatment). Scaffolds were cultured in 2 ml of medium for 6 weeks and half of the medium was changed every other day.

BIOCHEMICAL ANALYSIS AND IHC STAINING

Scaffolds were examined at weeks 0 and 6 for cell number, GAG and hydroxyproline content. Cell number was measured using a Picogreen assay (Molecular Probes; Eugene, OR, USA) with a conversion factor of 7.7 pg DNA/cell[38]. The GAG content was quantified by a dimethylmethylene blue (DMMB) dye assay (Biocolor; Newtownabbey, Northern Ireland)[38]. The hydroxyproline content was examined by a hydroxyproline assay (Accurate; Westbury, NY, USA)[38]. A conversion factor of 11.5 was determined in a preliminary study for converting hydroxyproline mass to collagen mass. Histological analysis was performed using a Safranin-O/fast green stain. IHC staining of types I and II collagen was performed in a Biogenex i6000 autostainer (San Ramon, CA, USA)[13]. The mouse monoclonal IgG anti-collagen I (1:1500 dilution) (Accurate Chemical; Westbury, NY, USA), mouse monoclonal IgG anti-collagen II (1:1000 dilution) (Chondrex; Redmond, WA, USA) and mouse monoclonal IgG anti-aggrecan (1:50 dilution) (Abcam; Cambridge, MA, USA) antibodies were chosen as primary antibodies. The streptavidin-linked horse anti-mouse IgG secondary antibody, ABC detection kit, and DAB (brown) and VIP (purple) substrate kits were obtained from Vector Laboratories (Burlingame, CA, USA). Double IHC staining of types I and II collagen was performed for sagittal sections of native ankle and TMJ tissues. Type I collagen staining was first conducted by following the above procedure with VIP as the substrate, avidin and biotin (Vector Laboratories) were then added in a blocking step to prevent the interaction between the first and second sets of labeling reagents, and type II collagen staining was finally performed with DAB as the substrate. Negative controls were prepared by omitting the primary antibodies, and the absence of non-specific staining was confirmed.

STATISTICAL ANALYSIS

All data were expressed as means ± one standard deviation and analyzed by an analysis of variance (ANOVA) followed by a Fisher's Protected Least Significance Difference *post hoc* test. Three-way ANOVAs with interaction were used to determine whether there were differences among time-points, different treatments, or cell types. A statistical threshold of $P < 0.05$ was used to indicate whether there were statistically significant differences among groups.

Results

CELL NUMBER

Over 6 weeks of culture, the ankle groups had significantly more cells than the TMJ groups ($P < 10^{-12}$) (Fig. 1). At week 0, there were 1.44 times more cells attached to scaffolds in ankle groups than in TMJ groups ($P < 0.05$), although two types of cells were seeded onto scaffolds at the same density (1.96 million cells per construct). At week 6, the average cell number of ankle groups was 1.63 million cells per construct, while the cell count of TMJ groups was 0.82 million cells per construct ($P < 10^{-12}$). With regard to IGF and glucosamine, ankle cells were more sensitive than TMJ cells. In all ankle groups, except for the glucosamine group, cell numbers increased from week 0 to week 6 ($P < 10^{-4}$), while cell numbers in TMJ groups did not exhibit any statistically significant differences from week 0 to week 6. In comparison to their respective controls, significant differences in cell numbers were not observed for either cell type. However, IGF and the combination of IGF and glucosamine in ankle groups promoted cell number compared to the glucosamine group ($P < 0.001$). In fact, the glucosamine groups had 15% and 13% decreases in cell number compared to the control for both the ankle and TMJ groups (not statistically significant), respectively.

GAG CONTENT

There was no significant difference in GAG content at week 0 between ankle and TMJ cells (Fig. 2). The average GAG production per construct of 107.4 μg in ankle groups was an order of magnitude higher than the average content of 11.3 μg per construct in TMJ groups after 6 weeks of culture ($P < 10^{-11}$). Ankle groups at week 6, with the exception of the glucosamine group, had drastically increased GAG production over time, as the IGF, combination and the control groups had 8.3 ($P < 10^{-9}$), 7.0 ($P < 10^{-8}$), and 4.1 ($P < 10^{-4}$) times higher GAG contents than at week 0, respectively, while there was no significant difference among TMJ groups. Both IGF and the combination promoted GAG production, with 1.79 and 1.49 times more GAGs in ankle groups than in the control ($P < 10^{-4}$ and 0.05, respectively), whereas glucosamine lessened the GAG production by 49% ($P < 0.01$).

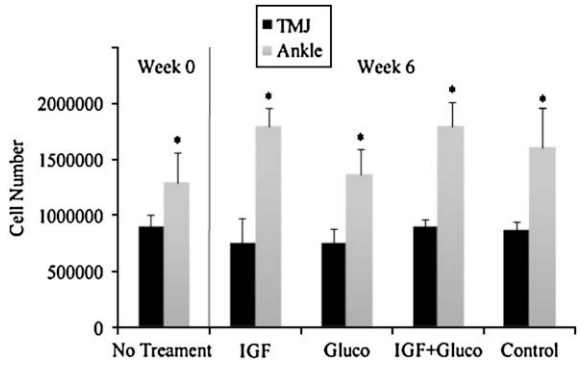

Fig. 1. Cell number per construct at weeks 0 and 6 ($n = 4$). Gluco denotes glucosamine. Symbol * means statistically significant difference between ankle groups and TMJ groups. Error bars represent standard deviations.

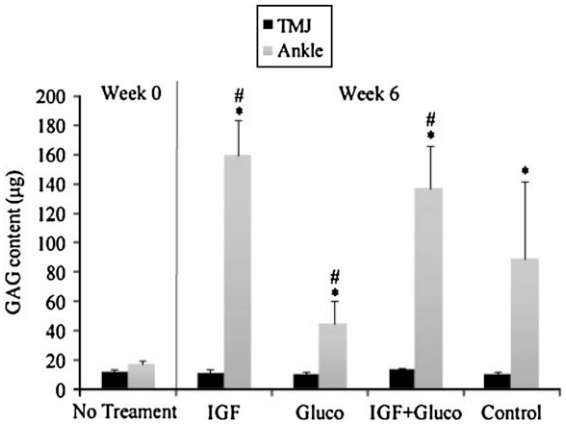

Fig. 2. GAG content per construct at weeks 0 and 6 ($n = 4$). Gluco denotes glucosamine. Symbol * means statistically significant difference between ankle groups and TMJ groups. Symbol # means statistically significant difference compared to the respective control group. Error bars represent standard deviations.

HYDROXYPROLINE CONTENT

As with cell number and GAG content, hydroxyproline content in ankle groups was significantly higher than in TMJ groups ($P < 10^{-11}$) (Fig. 3). Despite the fact that the ankle group had 5.8 times more hydroxyproline than the TMJ group at week 0, there was no statistically significant difference between them. In comparison to week 0, hydroxyproline content in ankle groups at week 6 increased over time by 8.9, 5.2, 8.0, and 6.0 times with the IGF group

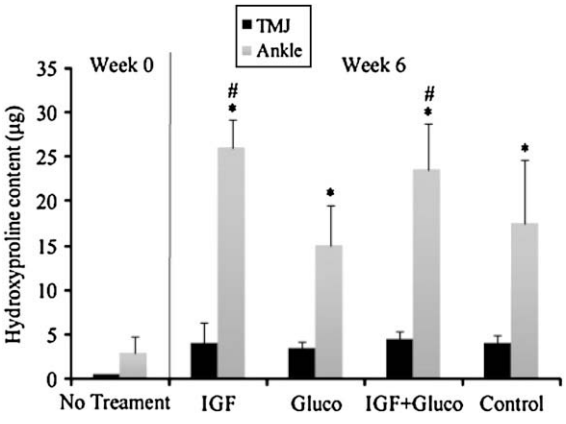

Fig. 3. Hydroxyproline content per construct at weeks 0 and 6 ($n = 4$) (multiply by a factor of 11.5 for collagen content). Gluco denotes glucosamine. Symbol * means statistically significant difference between ankle groups and TMJ groups. Symbol # means statistically significant difference compared to the respective control group. Error bars represent standard deviations.

($P < 10^{-9}$), the glucosamine group ($P < 10^{-4}$), their combination ($P < 10^{-8}$) and the control ($P < 10^{-5}$), respectively, while no significant differences were detected among TMJ groups. IGF and the combination in ankle groups had 26.0 and 24.0 μg of hydroxyproline per construct, which were 48% ($P < 0.005$) and 35% ($P < 0.05$) increases compared to the control, respectively.

IHC ANALYSES

At week 6, IHC analysis revealed a moderate staining of type I collagen and an intense staining of type II collagen in ankle groups (Fig. 4), with especially intense regions of collagen II staining in the IGF–glucosamine combination group. In the native ankle cartilage, type II collagen was dominant (Fig. 5). The constructs in TMJ groups demonstrated a significant presence of type I collagen and a minute amount of type II collagen (Fig. 4). It must be noted that the group combining IGF and glucosamine in the TMJ group demonstrated much more intense staining of type II collagen than all other TMJ groups. In native TMJ condylar cartilage, type I collagen was dominant in the superficial zone, abundant type I and moderate II collagen were observed in the proliferative zone, and type II collagen was dominant in the mature and hypertrophic zones (Fig. 5). In general, ankle cells had a weaker staining of type I collagen than TMJ cells and a stronger staining of type II collagen. IHC also revealed a more abundant presence of aggrecan in the IGF and the combination groups with ankle cells, compared to other ankle groups and all TMJ groups (Fig. 6), which was further confirmed by the GAG content from DMMB assays.

Discussion

In this study, mature porcine cartilages were used since the pig has been identified as a suitable model for TMJ tissue engineering strategies[2,39–42], although different species and ages[43] might lead to different results. The two types of cartilage cells were shown to differ *in vitro* in cell proliferation, GAG and collagen production, and collagen type. In addition, the results revealed that the compositions of tissue-engineered cartilages were different from their respective native tissues. Loading conditions may contribute to the different behaviors of the respective cell types, although it should be made clear that the TMJ is a load-bearing joint[42,44]. To the best of our knowledge, this was the first effort to compare cartilage cell sources for TMJ tissue engineering, as a first step in demonstrating that it may not be necessary to use cells from the same tissue when selecting a mature cell source for fibrocartilage regeneration. Moreover, this is the first example of collagens I and II double immunostaining of condylar cartilage, to the best of our knowledge.

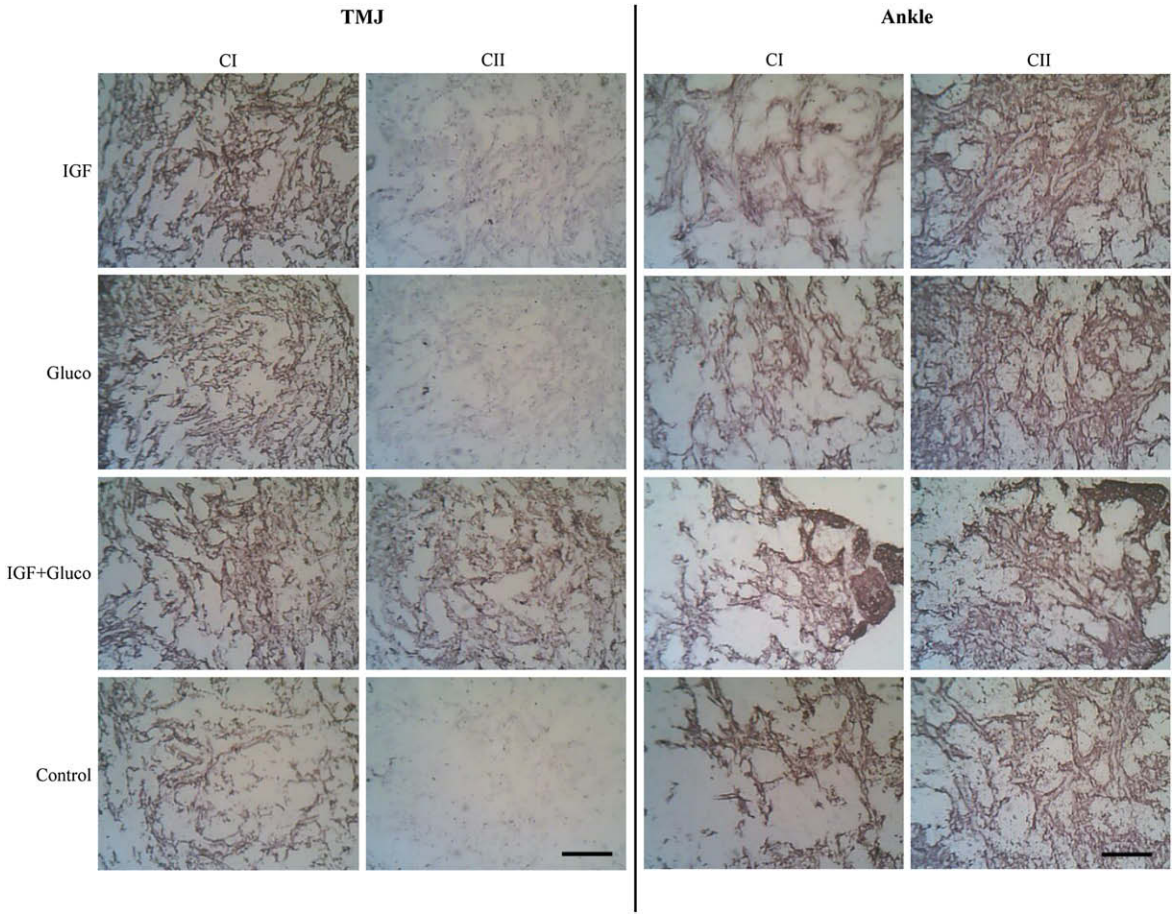

Fig. 4. IHC test for types I and II collagen at week 6 ($n = 2$). Positive staining is purple in color. The scale bar is 100 μm. CI = type I collagen and CII = type II collagen.

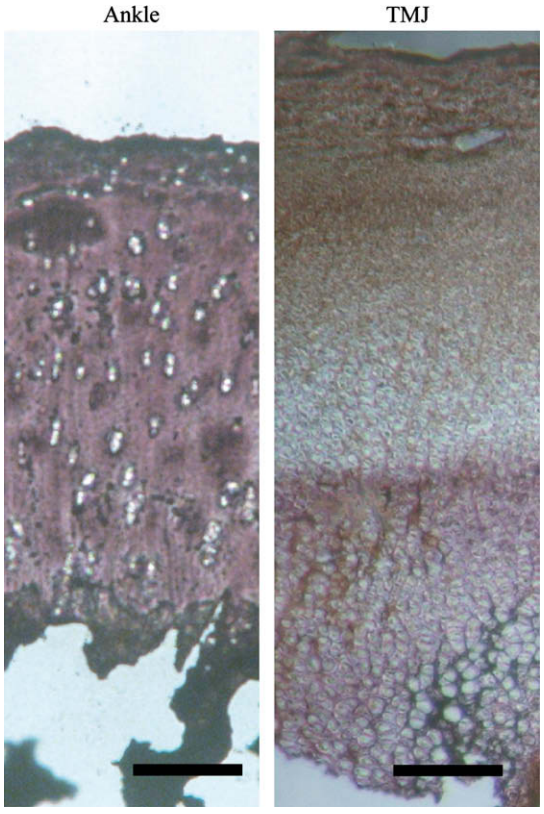

Fig. 5. Double IHC staining for types I and II collagen in sagittal sections from native tissues. Positive staining of type I collagen is brown in color. Positive staining of type II collagen is purple in color. Note that the hyaline cartilage is stained almost exclusively for type II collagen, whereas in sharp contrast the mandibular condylar cartilage consists of distinct zones dominated either by collagen I (superficial zone) or collagen II (mature and hypertrophic zones) staining. The scale bar is 100 μm.

Compared to hyaline cartilage cells, TMJ condylar cells were less proliferative in a 3D environment. The significant difference at week 0 in cell number between TMJ condylar cells and hyaline cartilage cells revealed that TMJ condylar cells had an inferior ability to attach onto PGA scaffolds compared to hyaline cartilage cells. This inferior attachment ability would likely explain the lower cellularity of TMJ groups at 6 weeks, given the superior proliferation rate of TMJ condylar cartilage cells in monolayer culture[45]. The exploration of new biomaterials may enhance the adhering ability of TMJ cells, such as Arg-Gly-Asp (RGD) modified poly(lactic-co-glycolic acid) (PLGA)[46] and poly-L-lactic acid (PLLA)[47]. During the 6 weeks of culture, TMJ condylar cells maintained a relatively constant cell number, indicating that TMJ condylar cartilage cells exhibited a behavior more similar to fibrocartilage cells such as TMJ disc cells and knee meniscus cells, although they were more proliferative in monolayer culture than ankle hyaline cartilage[45]. In fact, Almarza and Athanasiou[35] reported that *in vitro* culture of fibrocartilage cells (TMJ disc cells) even demonstrated a decrease in cell number over a 4-week period. Mesenchymal stem cells (human umbilical cord mesenchymal stromal cells) in a chondrogenic medium also had a decreased or stable cell number in PGA scaffolds over 4 weeks of culture in a fibrocartilage tissue engineering application[48]. The relatively invariable cellularity with TMJ condylar cells implied that a higher seeding density might be necessary.

TMJ condylar cells were inferior in extracellular matrix synthesis to hyaline cartilage cells. The ratios of GAG to collagen content in the TMJ groups were 0.25 on average, which is higher than the ratios in native tissues, while this ratio in the ankle groups was 0.47, which is comparable to native tissues[49–51]. The dominance of type I relative to type II collagen in TMJ groups (except for the combination of IGF and glucosamine) might be unexpected since there is abundant type II collagen present in the mature and hypertrophic zones of native TMJ condylar cartilage, though type I collagen produced by fibroblasts in the fibrous zone likely contributed to collagen production. A strong IHC staining of type II collagen and a moderate staining of type I collagen were observed in hyaline cartilage groups, indicating the formation of a fibrocartilage-like tissue, which is the goal with TMJ condylar cartilage regeneration. The abundant type I collagen in TMJ groups and moderate type I collagen presence in ankle groups together suggest the inevitable shift of TMJ and ankle cell phenotypes during *in vitro* culture, due to the differences between the *in vitro* and *in vivo* microenvironments, such as mechanical conditions, bioactive signals, and the exchange of nutrients and wastes. The dedifferentiated hyaline chondrocytes may be redifferentiated in an agarose gel[52], alginate beads[53], by use of high density culture[54], by substituting serum with the combination of TGF-β2 and IGF-I[55], or by coating a surface with chondrogenic molecules such as aggrecan[56,57]. However, it is still questionable whether redifferentiated chondrocytes have the ability to generate a properly assembled matrix[58].

TMJ condylar cells were less responsive to the stimulation of exogenous signals than hyaline cartilage cells, in that the IGF and IGF/glucosamine combination both significantly up-regulated collagen and GAG biosynthesis with hyaline cartilage cells, whereas no signals produced statistically significant differences for the TMJ cells compared to the control. It was unanticipated that glucosamine at 0.4 mM exhibited a noticeable inhibition of biosynthesis with ankle cartilage cells, although this concentration was the best for the up-regulation of GAG and collagen in our preliminary monolayer studies[28], indicating that further optimization of glucosamine concentrations in a 3D environment is required. However, Mroz and Silbert[59,60] also revealed that glucosamine chloride did not stimulate the formation of chondroitin sulfate with rat chondrocytes and even inhibited it at specific concentrations. Glucosamine is an important building block of proteoglycans in cartilage. However, a previous study[59] revealed that exogenous glucosamine did not participate in the pathway to produce GAGs, but rather played a role as a signal to regulate GAG and collagen production. Indeed, in the current study, the combination of IGF and glucosamine demonstrated a critical ability to retain type II collagen synthesis with TMJ condylar cells during *in vitro* culture, although the inherent mechanism needs to be further investigated in the future.

Ideally, tissue-engineered TMJ condylar cartilage should mimic the native zonal structure to achieve a functional condylar replacement. One strategy to accomplish this could be to separately harvest cells from the different zones of the condylar cartilage. However, the separation of different cell populations from their respective layers is limited by the accurate dissection of different layers of TMJ condylar cartilage due to the irregular zonal distribution. Countercurrent centrifugation, a technique extensively used with hematopoietic progenitor cells, was utilized in a previous study to elutriate five different fractions of cells from TMJ condylar cells based on the different sizes of cells[61]. Characterization of these cells suggested that fibroblast-like cells, mesenchymal

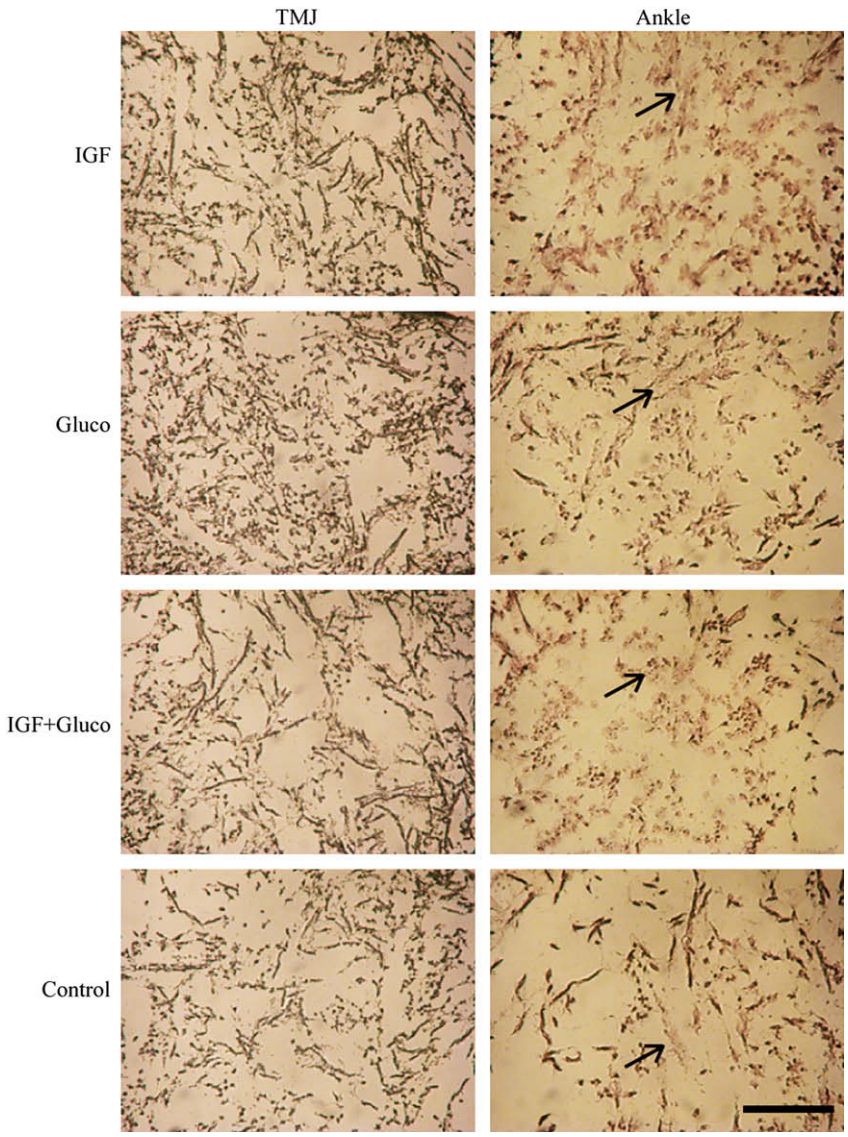

Fig. 6. IHC staining for aggrecan at week 6 ($n = 2$). Arrows indicate examples of positive staining of aggrecan (light purple color). The black color indicates PGA debris. The scale bar is 400 μm.

stem cells and hypertrophic cells might be identified from these five populations according to cell volume, alkaline phosphatase content, proteoglycan production, and collagen types. Seeding these cells into a stratified scaffold would be interesting but challenging work in the future.

In vitro culture of both types of cartilage cells appeared to exhibit a phenotypic shift during the 6 weeks of culture in PGA scaffolds. *In vitro* tissue engineering of TMJ condylar cartilage using mature cells produced a fibrous tissue with dominant type I collagen. The combination of IGF and glucosamine demonstrated the ability to maintain the expression of both types I and II collagen with TMJ condylar cartilage cells. *In vitro* culture of hyaline cartilage cells resulted in the fibrocartilage-like tissue genesis with both types I and II collagen. Most importantly, cell number and extracellular matrix content in the hyaline cartilage cell groups were significantly higher than in the TMJ condylar cartilage cell groups. Furthermore, from a clinical perspective, it is more reasonable to obtain cells from hyaline cartilage (or perhaps costal cartilage) than from degenerated TMJ condylar cartilage, in which the healthy cells are very limited, or from a healthy contralateral TMJ, which may lead to bilateral dysfunction. Therefore, given the importance of the superior biosynthetic activity by hyaline cartilage cells, and the clinical difficulty in obtaining donor cartilage, hyaline cartilage cells may be a more promising mature cell source than the TMJ itself for TMJ condylar cartilage tissue engineering, a principle which may also apply to other fibrocartilages such as the intervertebral disc and knee meniscus. In the future, experiments will include optimizing glucosamine concentrations in 3D culture, designing zonal scaffolds for seeding different populations of cells, and evaluating mechanical properties of tissue-engineered constructs for long-term culture ($>$6 weeks).

Conflict of interest

There are no conflicts of interest for any author.

Acknowledgments

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